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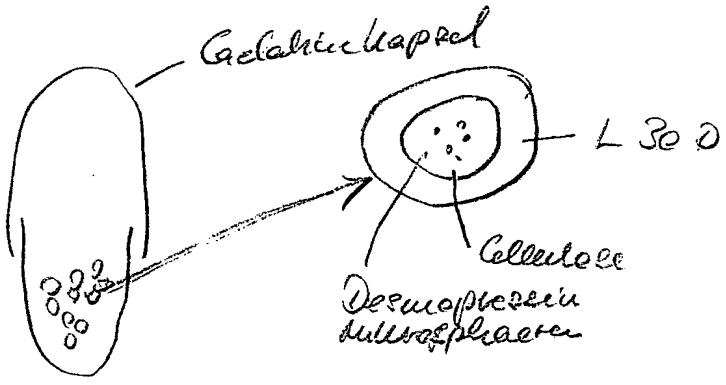
(54) Title: ORAL DOSAGE FORM OF DESMOPRESSIN (dDAVP)

(57) Abstract

dDAVP oral delivery systems are described wherein dDAVP retains good biological activity. In the preferred embodiment, dDAVP protein microspheres are made by emulsifying a solution of a protein such as zein and dDAVP with an immiscible solvent. Pellets containing the microspheres are prepared by an extrusion and spheronisation process, and are placed in a hard gelatin capsule. Release of biologically active dDAVP is achieved when the capsules are administered *in vivo* to cynomolgus monkeys.



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ORAL DOSAGE FORM OF DESMOPRESSIN (dDAVP)

Background of the Invention

This invention generally relates to polymeric microspheres, and pellets containing these microspheres, for oral delivery of Desmopressin.

Desmopressin (dDAVP) is a synthetic nonapeptide which is an analogue of the naturally occurring antidiuretic hormone, vasopressin, and is used to treat patients suffering from nocturnal enuresis, diabetes insipidus, hemophilia A, and Von Willebrand's disease. Due to the structural differences between dDAVP and vasopressin, dDAVP has greater antidiuretic activity, a more prolonged action, and reduced pressor activity. dDAVP is currently administered either as intravenous or subcutaneous injection or by intranasal administration. An oral dosage form is currently available Ferring (Malmo, Sweden), and consists of a compressed tablet formed of the peptide and standard pharmaceutical excipients, but has extremely low bioavailablity (less than 0.2%).

Oral delivery of peptides has been a major problem for many years. It is particularly a problem when the peptide to be delivered is unstable under the conditions encountered when administered to the patient, prior to reaching its targeted location. For example, it is preferable in many cases to administer

drugs orally, especially in terms of ease of administration, patient compliance, and decreased cost. However, many compounds are ineffective or exhibit low or variable potency when administered orally. In many cases this is because the drugs are unstable in the digestive tract or because they are inefficiently absorbed.

The field of oral drug delivery covers a broad range of delivery systems ranging from simple mechanical carriers such as compressed tablets to more complex delivery systems such as enteric coated products where the coating delays the release of an active compound until after passage through the stomach. A variety of enteric coatings have been used to protect therapeutic compounds from the low pH of the stomach and gastric enzymes prior to reaching the small intestine. In some cases these are effective. However, there are drugs that are also unstable to the conditions present in the small intestine. In such cases, if the drug is to be released in the small intestine, much higher dosages must be delivered for an effective amount to penetrate to the bloodstream.

Oral delivery at a desired rate and over a desired period is difficult to achieve. The conditions used to formulate the drug must not degrade the drug to be delivered, and the drug must not react with the drug matrix so as to inactivate or irreversibly bind the drug. Further, the delivery means must be stable to storage, and administrable using standard methodology. Other factors in drug delivery system design include ease in manufacture, use of readily available components, and consistency with respect to final composition and physical characteristics, including stability and release rate.

One means for orally administering a compound is to administer it within a polymeric matrix that releases compound as a function of polymer degradation and/or drug diffusion. In general, release is controlled by selection of the appropriate polymer, encapsulation conditions, drug loading and excipients.

A number of different systems have been proposed, most based on biodegradable natural or synthetic polymers, such as the natural polysaccharides, hydrophilic proteins such as albumin and hemoglobin, or polylactic acid, polyglycolic acid, polyorthoesters, or polyanhydrides, for example, as described for synthetic thermoplastic polymers in U.S. Patent Nos. 4,891,225 to Langer and 4,906,474 to Langer (polyanhydrides), 4,391,797 to Folkman, et al., (ethylenevinyl acetate polymers), and EPA 0 333 523 by The UAB Research Foundation and Southern Research Institute (polylactide-co-glycolide). Protein-based systems are described in U.S. Patent Nos. 4,925,673, 4,983,402, and 4,976,968 to Steiner and Rosen (protenoids) and PCT application WO 91/06287 by Enzytech (hydrophobic polymers such as zein).

A number of processes have been utilized to make microspheres, solid particles formed of a matrix having drug particles dispersed throughout, and microcapsules, particles formed of a core of drug or matrix containing drug which is surrounded by a layer of a second matrix material. Most microspheres are made of synthetic polymers, such as poly(lactic acid) or polyorthoesters, and are formed by solvent evaporation, spray drying, or phase separation. When the microspheres or microcapsules are used for drug delivery, the process must yield a product that is small, consistent in size and drug distribution, and with controlled degradation properties. R.C.

Oppenheim, Polymeric Nanoparticles and Microspheres, Guiot and Couvreur, eds., Ch.1, pp. 1-25 (CRC Press, 1986), review the formation, properties and drug delivery of protein microspheres. Most protein microspheres are crosslinked in solution using glutaraldehyde, or hardened at elevated temperatures. Unfortunately, these methods often result in significant loss of biological activity of incorporated materials, and lack of controlled size and *in vivo* degradation rates. For example, Suzuki, et al., Chem. Pharm. Bull. 37(4), 1051-1054 (1989), used the corn storage protein, zein, to prepare zein microspheres as carriers for chemotherapeutic agents by crosslinking a zein solution containing the drug. The resulting microspheres were heterogeneous in size, and incorporated less than 30% of the drug. This same group reported in Chem. Pharm. Bull. 37, 757-759 (1989), that yield and size range were improved by adding a catalytic amount of d,l-camphorsulfonic acid and rapidly adding polyvinylpyrrolidone, a surfactant and binder. Still, they incorporated less than 35% of the drug. PCT/US87/02025 by Clinical Technologies Associates, Inc., reports preparing and using microspheres for drug delivery made of "protenoids", by thermal condensation of mixed amino acids. EPO 158277 to Hoechst AG describes an implantable preparation for the controlled release of a peptide, buserelin, using zein as the carrier, formed by dissolving the peptide and the zein in alcohol, and spray drying and shaping the resulting mixture. EPO 077956 to Tanabe Seiyaku Ltd. describes using zein and other proteins as enteric coatings for microcapsules, formed using standard techniques for coating, i.e., spray coating or dipping. U.S. Patent No. 5,145,702 to Enzytech and Opta Food Ingredients, Inc. describes

microspheres formed by dissolving a hydrophobic protein in an organic solvent, then precipitating the protein in water. The resulting microspheres are very porous and are reported to be very effective as substitutes for fats in foods such as ice cream and mayonnaise. A useful method for formulating compounds in a non-crosslinked prolamine such as zein is described in U.S. Serial No. 07/902,505 entitled "Method for Producing Protein Microspheres" by Edith Mathiowitz, Howard Bernstein, Eric Morrel, and Kirsten Schwaller filed June 23, 1992, which is a continuation of 07/557,551 filed July 24, 1990, now abandoned, which is a continuation in part of 07/432,785 filed November 6, 1989, now abandoned.

With the exception of the latter method, none of the prior art methods yield a microparticle containing only a natural protein, having no binder or crosslinking agent present, which is suitable for oral administration of dDAVP. While the above systems are useful for many applications, they are not appropriate for some applications, such as orally administering drugs encapsulated in microspheres potentially capable of entering the gastro-intestinal tissue. A need exists for systems that can successfully deliver drugs orally with favorable release kinetics. Specifically, an oral formulation of dDAVP providing significant bioavailability would expand the patient populations able to benefit from this drug.

It is therefore an object of the present invention to provide methods for controlled or targeted oral drug delivery of dDAVP.

Summary of the Invention

Pellets containing microencapsulated dDAVP are described, which can be filled into capsules for oral administration of dDAVP to patients in need of treatment thereof. The dDAVP is first microencapsulated in a protein, preferably a hydrophobic protein such as zein, to produce microspheres less than about 100 micrometers, and preferably less than about ten to fifteen micrometers, in diameter. The microspheres are then formulated into pellets between approximately 0.1 mm and 5 mm in diameter, preferably spherical pellets approximately one mm in diameter, containing inert excipients, by an extrusion and spheronisation process, which are then enterically coated and packed into capsules.

Methods of orally administering the pellets to a patient in need of dDAVP treatment are also disclosed.

Detailed Description of the Invention

Biodegradable protein microspheres are formulated with inert excipients such as fillers, spheronization enhancers, disintegrants, surfactants and binders, to form pellets which are in turn enterically coated and filled into capsules, and are orally administered to a patient in need of treatment thereof for *in vivo* release of dDAVP.

As used herein, "micro" refers to a particle having a diameter of from about one nanometer to about 1000 micrometers. Microspheres are solid spherical particles formed of a matrix having drug particles dispersed throughout; microparticles are particles of irregular or non-spherical shape formed of matrix having drug particles dispersed therein.

Microcapsules are formed of a core of drug or matrix containing drug, surrounded by a layer of a second matrix material, which may contain drug dispersed therein. A microsphere or microcapsule may have an outer coating of a different composition than the material originally used to form the microsphere or microcapsule. Unless otherwise noted, the term microspheres can be used to encompass microcapsules and the term microparticles can be used to encompass microparticles, microspheres, and microcapsules. A "composite microsphere" is a microsphere formed of at least two different materials, either a protein and a polymer or two proteins. A pellet is an agglomerate, usually spherical, of microspheres and excipients, ranging in size from about 0.1 to about 5 mm.

As used herein, a "water-miscible organic solvent" is defined as an organic solvent which, at the temperature if interest, is completely miscible in water at concentrations of at least 40% by weight. Typical examples of water-miscible organic solvents include ethers, lower aliphatic (C_1-C_8) alcohols, lower aliphatic (C_2-C_8) ketones, dialkyl sulfoxides, such as dimethyl sulfoxide, dialkyl formamides, such as dimethyl formamide, and amides, such as acetamide.

dDAVP

dDAVP is obtained from a commercial source such as Diosynth (Ost, Netherlands) as a nine amino acid peptide provided in the monoacetate trihydrate form.

Proteins forming Microspheres

Proteins are used to make the microspheres since they are natural, offer a diversity of properties and are degraded *in vivo* into innocuous amino acids or small peptides. As used herein, proteins can be a single type of protein, a combination of proteins, or

a combination of protein with polymer. Hydrophobic proteins have limited solubility in water and are soluble in organic solvents, aqueous mixtures of organic solvents, and binary mixtures of organic solvents. Examples of useful proteins include prolamines, collagen, casein, and keratin.

Prolamines are characterized by having a large number of hydrophobic amino acids, such as glutamine, asparagine and proline. Prolamines are water-insoluble, but are soluble in many organic solvents, particularly alcohols, containing at least five percent (5%) water, but no more than sixty percent water, or a polar organic solvent.

Prolamines are readily available and inexpensive, for example, as the by-products of grain processing. Representative prolamines include gliadin, kafirin, zein and hordein. A preferred prolamine for use in making microspheres is zein. Both commercially available grades and purified forms of zein can be used. The properties of zein are described in detail by L.C. Swallen in: "Zein - A New Industrial Protein", Ind. and Eng. Chem., 33:394-398 (1941).

Preparation of Microspheres containing dDAVP

In the preferred embodiment described herein, protein microspheres are prepared by a phase separation, solvent removal process. The formation of the microspheres depends upon the differential solubility of proteins in water-miscible organic solvents, salt solutions, or acidic or basic solutions, as compared to their solubility in an immiscible phase, such as a nonpolar organic solvent or an oil. Most proteins are not soluble in oils.

Accordingly, protein is dissolved in a first solvent, which is a water-miscible organic, organic/aqueous, or binary organic solvent, acid, base

or salt solution (the encapsulating phase). The protein is "soluble" if more than about 0.5% (w/v) of the protein dissolves in the solvent to form a visually transparent solution at the temperature of interest. Prolamines are soluble, for example, in alcohols, such as ethanol; some ketones, such as methyl ethyl ketone and acetone; and amide solvents, such as acetamide; containing between about 5% and about 60% water; in extremely high (e.g., pH 10 or greater) or extremely low (pH 2 or less) pH solutions; and in aqueous solutions of from about 1 to about 6 N inorganic salts, such as NaCl and KBr.

A second solvent can be added in the zein solution to form a binary solvent for zein, solubilizing the zein. Many binary solvent systems for zein are known, in which the primary components are polyols, especially lower aliphatic alcohols, ketones, or glycols, and the secondary components are water, aromatic hydrocarbons, halogenated hydrocarbons, especially chlorinated hydrocarbons, nitroparaffins, aldehydes and cyclic ethers. Specific examples include mixtures of alcohols and halogenated hydrocarbons and mixtures of alcohols and propylene glycol with ethylene glycol.

Binary solvent systems for prolamines such as zein are reported by Manley and Evans, Ind. and Eng. Chem., 36, 661-665 (1943), hereby incorporated by reference.

The dDAVP, in the form of a solution or solid, is added to the protein solution. Typically, between about 0.1 and 20% (w/w) dDAVP is added, preferably between about 0.5 and 10% (w/w). The dDAVP/protein mixture is then introduced into a second liquid phase, the continuous phase, which is immiscible or of limited miscibility with the protein solvent and does not dissolve the protein. Solvents are "immiscible"

if they will not mix with each other to form a stable homogeneous solution at the operating temperature without mixing. Solvents are of "limited miscibility" if the second solvent does not form a stable homogenous solution with the protein solvent. Immiscible phases tend to form separate layers under these conditions. A vegetable oil is a preferred immiscible phase. Others include mineral oil, silicone oil, hexane, heptane, dodecane, and high boiling point petroleum ether.

One or more surfactants can be added to the protein/first solvent mixture or to the continuous phase to reduce the size of the protein microspheres. Suitable surfactants, and methods of use thereof, are known to those skilled in the art.

Vigorous agitation is applied to create droplets of the first solvent in the immiscible phase, and the first solvent is removed under conditions sufficient to form microparticles, for example, by evaporation or extraction. Efficient mixing can be achieved by fast mechanical stirring using a homogenizer and/or a baffled reactor. If necessary, the mixture can be heated to a temperature of from between 22°C and about 75°C, preferably between about 45°C and 55°C, for a period of between about 15 minutes and 45 minutes. If heated, the mixture is first cooled to room temperature, then the microspheres incorporating the compound are washed, separated from the mixture, and dried.

The microparticles can also be coated with protein or non-protein polymers. To make the coatings, protein or a non-protein polymer is first dissolved in a solvent; the microparticles to be coated are added to the solution; the protein or non-protein polymer/microparticle mixture is added to a second

liquid phase which is immiscible with the first solvent and a non-solvent for the protein or non-protein polymer coating; the mixture is agitated; and the first solvent is removed (usually by evaporation or extraction) under conditions sufficient to cause the microparticles to be coated with a protein or non-protein polymer coating.

The process described herein yields protein microspheres having a diameter of between about one nanometer and about 1000 micrometers, with an average diameter between 0.01 micrometer to less than about 10

Preparation of Pellets

Pelletization is a process that converts particles of drugs, such as microspheres, and excipients into small, free-flowing, spherical or semi-spherical units referred to as pellets. The most widely used pelletization processes are extrusion/spheronization, solution/suspension layering, and powder layering. Pellets generally range in size between about 0.5 and 2 mm.

Pelletized products offer significant therapeutic advantages over single-unit dosage forms, such as disintegrating tablets. Because they disperse freely in the gastrointestinal tract, they maximize drug absorption, reduce peak plasma fluctuations, and minimize potential side effects without appreciably lowering drug bioavailability. Pellets also reduce the effects of variable gastric emptying rates and overall transit times. Thus, patient to patient variability are minimized.

Pellets also provide flexibility in the design and development of oral dosage forms. For example, pellets composed of different drug entities can be blended and formulated in a single dosage form. Alternatively, pellets of different release rates of

the same drug can be combined in a single dosage form. Further, it is easier to adjust dosages within a single formulation if that formulation is comprised of pellets. Another advantage of pellets is the low surface area-to-volume ratio which provides an ideal shape for the application of film coatings.

The protein microspheres are produced for incorporation into pellets which make up the solid oral dosage form. The pellets may contain one or more inert excipients such as fillers, spheronization enhancers, disintegrants, surfactants and binders. These materials are known to those skilled in the art and are described in more detail below.

Methods of producing pellets include granulation, extrusion, and spheronization. A dry powder blend is produced including the desired excipients and microspheres. The dry powder is granulated with water or other non-solvents for microspheres such as oils and passed through an extruder forming "strings" or "fibers" of wet massed material as it passes through the extruder screen. The extrudate strings are placed in a spheronizer which forms spherical particles by breakage of the strings and repeated contact between the particles, the spheronizer walls and the rotating spheronizer base plate. The pellets are dried and screened to remove aggregates and fines.

Fillers

Fillers are water soluble or insoluble materials incorporated into the pellet formulation to add bulk. Types of fillers include sugars, starches and celluloses. The amount of filler in the formulation is in the range of between about 1 and about 90% by weight.

Spheronization Enhancers

Spheronization enhancers facilitate the production of spherical pellets. Substances such as zein, microcrystalline cellulose or microcrystalline cellulose co-processed with sodium carboxymethyl cellulose confer plasticity to the formulation as well as pellet strength and integrity. During spheronization, extrudates that are rigid, but not plastic, result in the formation of dumbbell shaped pellets and/or a high proportion of fines. Extrudates that are plastic, but not rigid, tend to agglomerate and form excessively large pellets. A balance between rigidity and plasticity must be maintained. The percent of spheronization enhancer in a formulation depends on the other excipient characteristics and is typically in the range of 10-90% (w/w).

Disintegrants

Disintegrants are substances which, in the presence of liquid, promote the disruption of the pellets. The function of the disintegrant is to counteract or neutralize the effect of any binding materials used in the formulation. The mechanism of disintegration involves, in large part, moisture absorption and swelling by an insoluble material. Examples of disintegrants include croscarmellose sodium and crospovidone which are typically incorporated into pellets in the range of 1-20% of total pellet weight. In many cases, soluble fillers such as sugars (mannitol and lactose) can also be added to facilitate disintegration of the pellets.

Surfactants

Surfactants may be necessary in pellet formulations to enhance wettability of poorly soluble or hydrophobic materials. Surfactants such as polysorbates or sodium lauryl sulfate are, if

necessary, used in low concentrations, generally less than 5%.

Binders

Binders are adhesive materials that are incorporated in pellet formulations to bind powders and maintain pellet integrity. Binders may be added as dry powder or as solution. Sugars and natural and synthetic polymers may act as binders. Materials added specifically as binders are generally included in the range of about 0.5%-15% w/w of the pellet formulation. Certain materials, such as microcrystalline cellulose, also used as a spheronization enhancer, also have additional binding properties.

Coatings

Various coatings can be applied to modify the properties of the pellets. Three types of coatings are seal, gloss and enteric. The seal coat prevents excess moisture uptake by the pellets during the application of aqueous based enteric coatings. The gloss coat improves the handling of the finished product. Water-soluble materials such as hydroxypropyl cellulose can be used to seal coat and gloss coat pellets. The seal coat and gloss coat are generally sprayed onto the pellets until an increase in weight between about 0.5% and about 5%, preferably about 1% for seal coat and about 3% for a gloss coat, has been obtained.

Enteric coatings consist of polymers which are insoluble in the low pH (less than 3.0) of the stomach, but are soluble in the elevated pH (greater than 4.0) of the small intestine. Polymers such as Eudragit®, RohmTech, Inc., Malden, MA, and Aquateric®, FMC Corp., Philadelphia, PA, can be used and are

layered as thin membranes onto the pellets from aqueous solution or suspension. The enteric coat is generally sprayed to a weight increase of about one to about 30%, preferably about 10 to about 15% and can contain coating adjuvants such as plasticizers, surfactants, separating agents that reduce the tackiness of the pellets during coating, and coating permeability adjusters. Other types of coatings having various dissolution or erosion properties can be used to further modify pellet behavior. Such coatings are readily known to one of ordinary skill in the art.

Capsule Filling

The pellets can be filled into standard pharmaceutical capsules, such as gelatin capsules, for ease of administration to a patient.

Methods of administering the capsules to a patient in need of dDAVP treatment

The capsules are orally administered to a patient in need of dDAVP treatment. The patient can be a human, or a non-human mammal such as equine, bovine, canine or feline.

An effective amount of a dDAVP formulation is that amount which will provide the desired therapeutic effect. The effective amount will be determined on an individual basis and will be based, at least in part, on consideration of the individual's size, the specific disease, the severity of symptoms to be treated and the result sought. Thus, the effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation. The active ingredient can be administered at once, or can be divided into a number of smaller doses to be administered at varying intervals of time.

The present invention will be further understood by reference to the following non-limiting examples.

Example 1: Production of Zein Microspheres Containing dDAVP

dDAVP was encapsulated in zein using an emulsification process. Zein, NF was from Freeman Industries, Inc. (Tuckahoe, NY) and Desmopressin Acetate B.P. was from Diosynth (Ost, Netherlands). A 15% (w/v) solution of zein was prepared by adding 600 mg of zein to 4 ml of 90% (v/v) solution of ethanol/water. The solution was mixed for approximately 15-20 minutes while being warmed to 36-38°C. Once the zein was dissolved, 32 mg of dDAVP was added to the zein solution such that the dDAVP was approximately 5% (w/w) of the total weight of zein and dDAVP. This mixture was stirred for 10-15 minutes at 36-38°C to dissolve the dDAVP. The solution was cooled to room temperature, and the pH was then adjusted to 4.4-4.6 with 1 N HCl. The pH-adjusted solution was warmed to 36-38°C in preparation for emulsification.

Prior to the emulsification process, 120 ml of corn oil was heated to 36-38°C. A homogenizer fitted with a fine emulsor screen was then completely immersed in the oil and turned on. When the homogenizer reached the appropriate speed, the warmed zein/dDAVP solution was poured, over a period of less than 5 seconds, into the oil. The mixture was homogenized for 15 seconds after all of the zein/dDAVP solution had been added.

This emulsion was immediately added to a vessel containing 250 ml of corn oil which was approximately twice the volume of the emulsion. Prior to adding the emulsion, the corn oil was preheated to 59-61°C and was continually mixed with an overhead mixer. After

adding the emulsion, the mixture was maintained at 50-52°C for 25 minutes while being stirred at 200 rpm. The mixture was then allowed to cool until the batch reached 38°C. Mixing was continued during the cooling. The partially cooled mixture was then stored overnight at 4°C.

The microspheres were separated from the corn oil using vacuum filtration and were washed with diethyl ether. The washing continued until the ether filtrate was clear. The microspheres were finally filtered to form a nearly dry cake and were transferred to a vacuum desiccator for overnight drying.

The microspheres produced were sized using a Coulter Multisizer and had a mean diameter of 4.0 micrometers. The microspheres had a dDAVP load of 85 to 115% of theoretical load (about 5% (w/w)) and a moisture content of 1.7 % (w/w).

Example 2: Production of Pellets containing Zein microspheres.

Zein microspheres containing dDAVP were incorporated into approximately 1 mm pellets by an extrusion and spheronization process.

To produce about 500 g of uncoated pellets, the following components were premixed: microcrystalline cellulose, NF Grade PH 101 (Avicel PH 101 or equivalent), lactose, NF, croscarmellose sodium, NF (Ac-Di-Sol or equivalent) and milled zein, NF. The milled zein is present in the formulation for two reasons: 1) zein has good spheronizing properties and 2) the milled zein approximates the physicochemical characteristics of the microspheres allowing for dosage flexibility within the formulation. Pellets have been manufactured containing up to 42% of milled zein.

The composition of the inert excipient mixture was as follows for a 500 gram batch of uncoated pellets:

	(grams)
Zein NF milled	20.0
Microcrystalline cellulose NF (type PH101)	250.0
Lactose NF	175.0
Croscarmellose Sodium NF	50.0

The components were blended in a Ziploc™ bag for five minutes. Blending was achieved by sealing the bag with air inside and continuously turning the bag by hand.

Five grams of dDAVP microspheres were dispensed into a clean Ziploc™ bag. The dDAVP microspheres were serially diluted by adding aliquots of the excipient blend described above to the bag containing the microspheres. The amount of each aliquot was approximately equal to the amount of material in the bag containing the dDAVP microspheres so that each serial dilution was approximately 1:1. Each aliquot addition was followed by three minutes of blending. Again, this was accomplished by sealing the bag with air inside and continuously turning the bag by hand. The material was blended for a further twenty minutes after adding the final aliquot.

The bulk dry powder blend, described above, was granulated by transferring it into a planetary type mixer and adding purified water. The amount of water added was calculated based on the exact weight of the dry powder blend available (1.08 grams of water per gram of dry powder blend). The water was added while

mixing over a period of two minutes; mixing was then continued for an additional one minute.

The extruder (Nica E-140 or equivalent) was assembled with a 1.2 mm screen. The extruder was switched on, the feeder speed was set at 50 rpm, and the head speed was set at 70 rpm. The wet granulated powder mass was emptied into the extruder hopper and extruded directly onto a stainless steel tray. The extrudate collected on the tray was then transferred into the spheronizer (Nica S-450 or equivalent) and processed at a speed of 450 rpm with a spheronization time of 2 minutes and an evacuation time of 1.5 minutes. The spheronizer was started after the extrudate was charged onto the stationary spheronizing plate.

The pellets formed by this process were collected from the spheronizer discharge port into a polyethylene bag and were transferred into a fluid bed processor. The fluid bed processor (Niro, Inc. Aeromatic MP-1 or equivalent) was assembled with a stainless steel outer container, an 8% distribution plate, a 100-mesh container screen, and a screen for exhaust filters.

The pellets were dried for 60 minutes with an inlet temperature of 40°C and a pressure drop across the distribution plate of 150-170 mm H_2O. The air flow in the MP-1 was adjusted and monitored to produce satisfactory fluidization of the material. Following the drying period the heater was switched off, and the fluidized material was cooled for at least 5 minutes until the exhaust temperature was 30°C. The pellets were harvested and sieved through stacked U.S. Standard No. 16 and No. 30 sieves to produce three fractions: greater than 1.2 mm in diameter, less than

0.6 mm in diameter, and between 1.2 and 0.6 mm in diameter. The pellets with diameters in the range of 0.6 to 1.2 mm (-16 +30 fraction) were retained for coating.

Coating of Pellets

Following drying and sieving, the pellets were coated with a seal coat and an enteric coat. For the seal and enteric coating procedure, the MP-1 processor was equipped with a stainless steel outer container, a 4% Aerocoat distribution plate with 15 mm coating column gap, a 1.0 mm orifice Schlick nozzle, a drying container insert and an exhaust filter screen. The seal coat solution was prepared by mixing Opadry® Colorcon, Inc., West Point, PA, into purified water using a magnetic stirrer. The seal coat solution was and must be used within 24 hours of its preparation. Ideally 1000 grams, but not less than 800 grams, of the 0.6-1.2 mm diameter uncoated pellets were fluidized in the processor with 100-120 pascals air flow, 1.4 bar atomizing air and an inlet temperature of 60°C.

The seal coat solution was sprayed at a rate of 10.0 grams of solution per minute for 21 minutes. Once the appropriate amount of seal coat (10 grams per 1000 grams uncoated pellets) had been applied, the inlet air temperature was decreased and the outlet air temperature was monitored.

The enteric coating suspension for 1000 grams of uncoated pellets was prepared by mixing 500 grams of Eudragit L 30 D suspension with 15 grams triethyl citrate for 45 minutes using an overhead mixer with a propeller-type blade. Fifteen grams of Talc and 662.5 grams of purified water were added to the suspension and mixing was continued for thirty minutes and until coating was completed. The enteric coating suspension

was prepared no more than eight hours before use. The enteric coating suspension was sprayed onto the pellets at an initial spray rate of 7.4 grams per minute. The inlet air temperature was adjusted to maintain the outlet air temperature at 35°C throughout the enteric coating process. After five minutes of coating at 7.4 grams per minute, the spray rate was increased to 10.5 grams per minute and was maintained at this rate for the remainder of the coating process. When sufficient enteric coating material (182.5 grams solids per 1000 grams uncoated pellets) was applied, the coated pellets were dried at the same process settings for an additional four minutes. The processor heat was then turned off, and the pellets were cooled until the outlet air temperature reached 30°C.

Pellets were filled by hand into size 1, clear, colorless, hard gelatin capsules.

Example 3: Administration of Capsules to Cynomolgus Monkeys to demonstrate *in vivo* efficacy.

The capsules containing the enteric coated pellets which were produced in Example 2 were administered orally to six cynomolgus monkeys in a two way crossover study to evaluate the pharmacodynamic activity of the formulation. Half the monkeys were randomly assigned to a placebo group and the other half to the active dosage form. Following an overnight fast, animals were given a 2% water load based on their body weights and then orally administered either a placebo capsule or an active capsule containing 80 μg of dDAVP. Urine samples were collected at 15 minute intervals, either until 50% of the water load had been excreted or for approximately 8 hours. The treatments were reversed after a washout

period of 72 hours. Analysis of urine collection data revealed five of the six animals exhibited a delay in the time to excrete 50% of the given water load when receiving the capsules containing dDAVP as compared to receiving a placebo capsule. The times to excrete 50% of the water loads for each animal are summarized in Table 1. Five of the six animals had a significant delay in excreting 50% of the water load.

Table 1: Effect of dDAVP on Urinary excretion in Animals

Time to Excrete 50% Water Load (hours)

Monkey ID	Placebo	dDAVP Capsule
545X	1.4	3.3
A530	1.5	3.0
111-379	1.1	3.6
VW1211X	1.4	4.1
VW1212X	2.2	3.4
111-10	1.4	1.6

Modifications and variations of the present invention, compositions and methods for making microspheres, pellets and capsules containing dDAVP, and methods for administering the microsphere-containing pellets to a patient, will be obvious to those skilled in the art from the foregoing detailed description of the invention. Such modifications are intended to come within the scope of the appended claims.

We claim:

1. A protein microsphere having a diameter of between 0.01 micrometers and 100 micrometers, containing dDAVP in a pharmaceutically effective amount.

2. The microsphere of claim 1 wherein the concentration of dDAVP is in a concentration of between 0.1% and 20% by weight.

3. The microsphere of claim 1 formed from a protein selected from the group consisting of prolamine, collagen, casein, keratin, and mixtures thereof.

4. The microsphere of claim 3 wherein the prolamine is zein.

5. The microsphere of claim 1 wherein the diameter is less than 10 micrometers.

6. The microsphere of claim 1 formulated in a pellet comprising material selected from the group consisting of a surfactant, coating, filler, disintegrant, spheronization enhancer, and binder.

7. The microsphere of claim 6 further wherein the pellet is filled into a capsule.

8. The pellet of claim 6 wherein the diameter of the pellet is between about 0.1 to about 5 mm.

9. The pellet of claim 6 wherein the material comprises between about 1 and about 90% of the total pellet weight.

10. The pellet of claim 6 wherein the filler forming the pellet is selected from the group consisting of sugars, starches and celluloses, further comprising a surfactant; a binder; a disintigrant, and a spheronizing enhancer.

11. The pellet of claim 6 wherein the disintegrant is present in a range of between about 1 and about 20% of the total pellet weight.

12. The pellet of claim 6 wherein the surfactant is present in a range of between greater than about 0 and about 5% of the total pellet weight.

13. The pellet of claim 6 wherein the binder is present in a range of between about 0.5 and about 15% of the total pellet weight.

14. The pellet of claim 6 wherein the spheronizing enhancer is present in a range of between about ten and ninety percent by weight.

15. The pellet of claim 6 wherein the filler is present in a range of between about 1% and about 90%.

16. The pellet of claim 6 wherein the coating is a seal coating.

17. The pellet of claim 6 wherein the coating is an enteric coating.

18. A method for producing protein microspheres comprising the steps of:

(a) contacting a protein solution, wherein the protein solution contains at least one type of protein and dDAVP, with a second liquid, wherein the second liquid is of limited miscibility with the protein solvent, in a ratio of protein solution to second liquid of at least 1:20 to form a protein-non-solvent mixture;

(b) agitating the protein-non-solvent mixture to form a dispersion of the protein solution in the second liquid; and

(c) removing the protein solvent to form stable protein microspheres without crosslinking or heat denaturation.

19. The method of claim 18 wherein the microspheres are formed at a temperature of less than about 75°C.

20. The method of claim 18 wherein the protein is selected from the group consisting of prolamine, collagen, casein, keratin, and mixtures thereof.

21. The method of claim 20 wherein the prolamine is zein.

22. The method of claim 18 wherein the immiscible solvent is selected from the group consisting of vegetable oil, mineral oil and silicone oil.

23. The method of claim 18 further comprising formulating the microspheres in pellets for suitable for oral administration of dDAVP.

24. The method of claim 23 further comprising coating the pellets with a coating selected from the group consisting of enteric coatings and seal coatings and gloss coatings.

25. The method of claim 23 further comprising filling a capsule with the pellets.

26. A method for administering dDAVP comprising orally administering protein microspheres having a diameter of between 0.01 micrometers and 100 micrometers, containing dDAVP in a therapeutically effective amount.

27. The method of claim 26 wherein the dDAVP is in a concentration of between 0.1% and 20% by weight.

28. The method of claim 26 wherein the microspheres are formulated in pellets.

29. The method of claim 28 wherein the microspheres are formed into pellets which are filled into a capsule.

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 95/00275

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/51 A61K9/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 06286 (ENZYTECH INC.) 16 May 1991 cited in the application see page 6 - page 7 ---	18-22
A	WO,A,93 13753 (ALFATEC PHARMA GMBH) 22 July 1993 ---	
A	J. PHARM. PHARMACOL., vol. 46,no. 1, 1994 pages 8-13, M.S. LATHA ET AL 'Glutaraldehyde cross-linked bovine casein microsperes as a matrix for the controlled release of theophylline: in vitro studies.' -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte: ›nal Application No
PCT/US 95/00275

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